(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018675 A1

(51) International Patent Classification[7]: C12N 15/11, A61K 31/712

(21) International Application Number: PCT/CA2003/001276

(22) International Filing Date: 21 August 2003 (21.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/405,193	21 August 2002 (21.08.2002)	US
60/408,152	3 September 2002 (03.09.2002)	US
60/319,748	2 December 2002 (02.12.2002)	US
60/472,387	20 May 2003 (20.05.2003)	US

(71) Applicants *(for all designated States except US)*: **THE UNIVERSITY OF BRITISH COLUMBIA** [CA/CA]; #103 - 6190 Agronomy Road, Vancouver, British Columbia V6T 1Z3 (CA). **GLEAVE, Martin, E.** [CA/CA]; 4693 Drummond Drive, Vancouver, British Columbia V6R 1E8 (CA).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **JANSEN, Burkhard** [AT/CA]; 401 - 1631 Vine Street, Vancouver, British Columbia V6K 3J3 (CA).

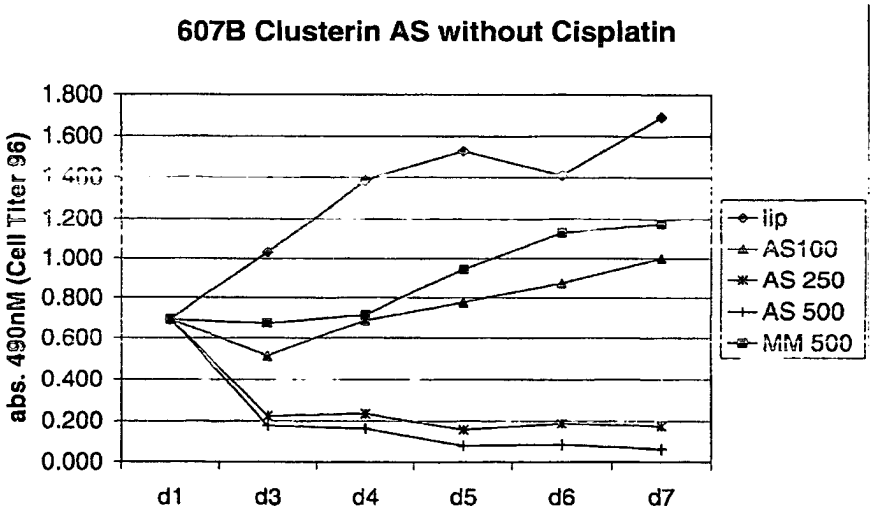
(74) Agents: **KINGWELL, Brian, G.** et al.; Fetherstonhaugh & Co. 2200-650 West Georgia Street, Box 11560, Vancouver, British Columbia V6B 4N8 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,

[Continued on next page]

(54) Title: TREATMENT OF MELANOMA BY REDUCTION IN CLUSTERIN LEVELS



(57) Abstract: Treatment of melanoma is achieved through reduction in the effective amount of clusterin in melanoma cells. Thus, in accordance with one aspect of the invention, there is provided a method for treatment of melanoma in a mammalian subject, preferably a human, comprising the step of administering to the subject a therapeutic agent effective to reduce the effective amount of clusterin in the melanoma cells. The therapeutic agent may be, for example, an antisense ODN or small inhibitory RNA (siRNA) compound targeted to clusterin. The present invention also provides a method for regulating expression of bcl-xL in a subject or cell line comprising administering to the subject or cell line an agent effective to modulate the amount of clusterin expression. In particular, in clusterin expressing cells, the expression of bcl-xL is down-regulated when the effective amount of clusterin is reduced. Such inhibition is significant because bcl-xL is known to act as an inhibitor of apoptosis.

WO 2004/018675 A1

ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Treatment of Melanoma by Reduction in Clusterin Levels

DESCRIPTION

This application claims the benefit and priority of US Provisional Applications Nos. 60/405,193 filed August 21, 2002, 60/408,152 filed September 3, 2002, 60/319,748 filed December 2, 2002, and 60/472,387, filed May 20, 2003 all of which are incorporated herein by reference in jurisdictions permitting such incorporation.

Background of the Invention

This application relates to antisense treatments for melanoma by inhibition of clusterin, also known as testosterone-repressed prostate message-2 (TRPM-2), for example by the administration of antisense oligonucleotides specific for clusterin.

Clusterin or TRPM-2 is a ubiquitous protein, with a diverse range of proposed activities. In prostate epithelial cells, expression of Clusterin increases immediately following castration, reaching peak levels in rat prostate cells at 3 to 4 days post castration, coincident with the onset of massive cell death. These results have led some researchers to the conclusion that clusterin is a marker for cell death, and a promoter of apoptosis. On the other hand, the observation that Sertoli cells and some epithelial cells express high levels of clusterin without increased levels of cell death, raises questions as to whether this conclusion is correct. Sensibar et al., *Cancer Research* 55: 2431-2437 (1995) reported on *in vitro* experiments performed to more clearly elucidate the role of clusterin in prostatic cell death. They utilized LNCaP cells transfected with a gene encoding clusterin and observed whether expression of this protein altered the effects of tumor necrosis factor α (TNFα), to which LNCaP cells are very sensitive, with cell death normally occurring within about 12 hours. Treatment of the transfected LNCaP cells with TNFα was shown to result in a transient increase in clusterin levels for a period of a few hours, but these levels had dissipated by the time DNA fragmentation preceding cell death was observed. Using an antisense molecule corresponding to the bases 1-21 of the clusterin sequence, but not other clusterin antisense oligonucleotides, resulted in a substantial reduction in expression of clusterin, and an increase in apoptotic cell death in LNCaP cells exposed to TNFα. This led Sensibar et al. to the hypothesis that overexpression of clusterin could protect cells from the cytotoxic effect of

TNF, and that clusterin depletion is responsible for the onset of cell death, although the mechanism of action remains unclear.

PCT Publication WO00/049937, which is incorporated herein by reference in all jurisdictions permitting such incorporation, describes the use of antisense therapy which reduces the expression of clusterin to provide therapeutic benefits in the treatment of cancer of prostate cancer, renal cell cancer and some breast cancers. Furthermore, combined use of antisense clusterin plus cytotoxic chemotherapy (e.g. taxanes) synergistically enhances chemosensitivity in hormone refractory prostate cancer. Radiation sensitivity is also enhanced when cells expressing clusterin are treated with antisense clusterin oligodeoxynucleotides (ODN).

Summary of the Invention

The present application relates to the treatment of melanoma through reduction in the effective amount of clusterin. Thus, in accordance with one aspect of the invention, there is provided a method for treatment of melanoma in a mammalian subject, preferably a human, comprising the step of administering to the subject a therapeutic agent effective to reduce the effective amount of clusterin in the melanoma cells. The therapeutic agent may be, for example, an antisense ODN or small inhibitory RNA (siRNA) compound targeted to clusterin.

The present invention also provides a method for regulating expression of bcl-xL in a subject or cell line comprising administering to the subject or cell line an agent effective to modulate the amount of clusterin expression. In particular, in clusterin expressing cells, the expression of bcl-xL is down-regulated when the effective amount of clusterin is reduced. Such inhibition is significant because bcl-xL is known to act as an inhibitor of apoptosis. See for example US Patent No. 6,172,216 which is incorporated herein by reference to in those jurisdictions where such incorporation is allowed.

Brief Description of the Drawings

Fig. 1 shows the results when 607B melanoma cells were treated with either the antisense oligonucleotide at concentrations of 100, 250 or 500 nM, or a scrambled mismatch control at a concentration of 100 nM on two consecutive days.

Fig. 2 provides a graphic representations of clusterin expression in 518A2 cells after treatment with cisplatin and either an antisense oligonucleotide or a scrambled, mismatch control.

Fig. 3 shows cell survival of Mel Juso melanoma cells stably transfected with either an empty control vector (Neo) or a vector directing overexpression of clusterin were grown in medium containing 10 μM cisplatin.

Description of the Invention

As used in the specification and claims of this application, the term "clusterin" refers to the glycoprotein originally derived from rat testes, and to homologous proteins derived from other mammalian species, including humans, whether denominated as clusterin or an alternative name. The sequences of numerous clusterin species are known. For example, the sequence of human clusterin is reported by Wong et al., *Eur. J. Biochem.* 221 (3), 917-925 (1994), and in NCBI sequence accession number NM_001831 and is set forth in the Sequence Listing as Seq. ID. No. 1. In this sequence, the coding sequence spans bases 48 to 1397.

The present invention provides a therapeutic composition, and methods for using such a composition for treatment of melanoma, particularly in humans. The therapeutic compositions and methods of the invention achieve a reduction in the effective amount of clusterin present in the individual being treated. As used in this application, the "effective amount of clusterin" is the amount of clusterin which is present in a form which is functional to provide anti-apoptotic protection. The effective amount of clusterin may be reduced by decreasing the expression rate of clusterin, increasing the rate of clusterin degradation, or by modifying clusterin (for example by binding with an antibody) such that it is rendered inactive.

Antisense ODN Therapeutics

In one embodiment of the invention, reduction in the effective amount of clusterin may be accomplished by the administration of antisense ODNs, particularly antisense ODNs which are complementary to a region of the clusterin mRNA spanning either the translation initiation site or the termination site. Exemplary sequences which can be

employed as antisense molecules in the method of the invention are disclosed in PCT Patent Publication WO 00/49937, US Patent Publication US-2002-0128220-A1, and US Patent No. 6,383,808, all of which are incorporated herein by reference in those jurisdictions where such incorporation is permitted. Specific antisense sequences are set forth in the present application as Seq. ID Nos.: 2 to 19.

The ODNs employed may be modified to increase the stability of the ODN *in vivo*. For example, the ODNs may be employed as phosphorothioate derivatives (replacement of a non-bridging phosphoryl oxygen atoms with a sulfur atom) which have increased resistance to nuclease digestion. MOE (2'-O-(2-methoxyethyl) modification (ISIS backbone) is also effective. Construction of such modified ODN is described in detail in US Patent Application 10/080,794 which is incorporated herein by reference in those jurisdictions permitting such incorporation. A particularly preferred composition is a 21mer oligonucleotide (cagcagcagagtcttcatcat; SEQ ID NO: 4) targeted to the translation initiation codon and next 6 codons of the human clusterin sequence (Genbank accession no: NM_001831) with a 2'-MOE modification. This oligonucleotide has a phosphorothioate backbone throughout. The sugar moieties of nucleotides 1-4 and 18-21 (the "wings") bear 2'-O-methoxyethyl modifications and the remaining nucleotides (nucleotides 5-17; the "deoxy gap") are 2'-deoxynucleotides. Cytosines in the wings (i.e., nucleotides 1, 4 and 19) are 5-methylcytosines.

Administration of antisense ODNs can be carried out using the various mechanisms known in the art, including naked administration and administration in pharmaceutically acceptable lipid carriers. For example, lipid carriers for antisense delivery are disclosed in US Patents No. 5,855,911 and 5,417,978 which are incorporated herein by reference. In general, the antisense is administered by intravenous, intraperitoneal, subcutaneous or oral routes, or direct local tumor injection.

The amount of antisense ODN administered is one effective to inhibit the expression of Clusterin in melanoma cells. It will be appreciated that this amount will vary both with the effectiveness of the antisense ODN employed, and with the nature of any carrier used. The determination of appropriate amounts for any given composition is within the skill in the art, through standard series of tests designed to assess appropriate therapeutic levels.

RNAi Therapeutics

Reduction in the effective amount of clusterin can also be achieved using RNAi therapy. RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire et al. (1998) Nature 391, 806-811, incorporated herein by reference). dsRNA directs gene-specific, post-transcriptional silencing in many organisms, including vertebrates, and has provided a new tool for studying gene function. RNAi involves mRNA degradation, but many of the biochemical mechanisms underlying this interference are unknown. The use of RNAi has been further described in Carthew et al. (2001) Current Opinions in Cell Biology 13, 244-248, and Elbashir et al. (2001) Nature 411, 494-498, both of which are incorporated herein by reference.

In the present invention, isolated RNA molecules mediate RNAi. That is, the isolated RNA molecules of the present invention mediate degradation or block expression of mRNA that is the transcriptional product of the gene, which is also referred to as a target gene. For convenience, such mRNA may also be referred to herein as mRNA to be degraded. The terms RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) may be used interchangeably to refer to RNA that mediates RNA interference. These terms include double-stranded RNA, single-stranded RNA, isolated RNA (partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA), as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the RNA or internally (at one or more nucleotides of the RNA). Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAi molecules are referred to as analogs or analogs of naturally-occurring RNA. RNA of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate RNAi. As used herein the phrase "mediate RNAi" refers to and indicates the ability to distinguish which mRNA are to be affected by the RNAi machinery or process. RNA that mediates RNAi interacts with the RNAi machinery such that it directs the machinery to degrade particular mRNAs or to otherwise reduce the expression of the target protein. In one embodiment, the present invention relates to RNA

molecules that direct cleavage of specific mRNA to which their sequence corresponds. It is not necessary that there be perfect correspondence of the sequences, but the correspondence must be sufficient to enable the RNA to direct RNAi inhibition by cleavage or blocking expression of the target mRNA.

As noted above, the RNA molecules of the present invention in general comprise an RNA portion and some additional portion, for example a deoxyribonucleotide portion. The total number of nucleotides in the RNA molecule is suitably less than 49 in order to be effective mediators of RNAi. In preferred RNA molecules, the number of nucleotides is 16 to 29, more preferably 18 to 23, and most preferably 21-23. Suitable sequences are set forth in the present application as Seq. ID Nos. 20 to 43.

The siRNA molecules of the invention are used in therapy to treat patients, including human patients, that have cancers or other diseases of a type where a therapeutic benefit is obtained by the inhibition of expression of the targeted protein. siRNA molecules of the invention are administered to patients by one or more daily injections (intravenous, subcutaneous or intrathecal) or by continuous intravenous or intrathecal administration for one or more treatment cycles to reach plasma and tissue concentrations suitable for the regulation of the targeted mRNA and protein.

Additional therapeutic agents

The method for treating melanoma in accordance with the invention may further include administration of chemotherapy agents or other agents useful in melanoma therapy and/or additional antisense ODNs directed at different targets in combination with the therapeutic effective to reduce the amount of active clusterin. For example, antisense clusterin ODN increases sensitivity to conventional chemotherapy agents such as taxanes (paclitaxel or docetaxel), mitoxanthrone, and gemcitabine. Other agents likely to show synergistic activity include other cytotoxic agents (e.g. cyclophosphamide, decarbazine, topoisomerase inhibitors), angiogenesis inhibitors, differentiation agents and signal transduction inhibitors. Similarly, combinations of clusterin antisense with other antisense species such as antisense Bcl-2, Bcl-xl and c-myc ODN to provide greater effectiveness.

Method of regulating Bcl-xL expression

While chaperone-like function has been proposed for the clusterin protein, the specific molecular mechanism responsible for clusterin's role in apoptosis remains elusive. In the human melanoma cell line that expressed clusterin at a very low levels, over-expression of clusterin by stable transfection not only led to a marked increase in resistance to a cytotoxic treatment (Figure 3), but led also to an up-regulation of the anti-apoptotic bcl-2 family member bcl-xL as shown by Western blotting. Conversely treatment of clusterin-expressing melanoma cells led to a marked down-regulation of bcl-xL thus providing a possible mechanism for the antiapoptotic potency of clusterin. Neither clusterin overexpression by transfection nor clusterin antisense treatment altered the expression of other Bcl-2 family members tested in human melanoma cells. Thus, clusterin regulates the anti-apoptotic bcl-2 family member bcl-xL. Such inhibition is significant because bcl-xL is known to act as an inhibitor of apoptosis (See US Patent No. 6,182,216 which is incorporated herein by reference in those jurisdictions permitting such incorporation).

The invention will now be further described with reference to the following, non-limiting examples.

Example 1

Expression of clusterin in two different batches of normal human melanocytes (NHEM 6083 and 2489) and four human melanoma cell lines (518A2, SKMEL-28, Mel-Juso and 607B). Cells were grown in 6 cm dishes and harvested when they were 80-90% confluent. 30:g of protein per lane was applied onto a 10% SDS-Page gel and probed with a polyclonal goat anti-clusterin antibody. Panceau red stain and an antibody directed against ß-actin were used as a loading control. In each case, the antisense inhibitor of clusterin used is based on the advanced antisense chemistry 2'MOE as described in US Patent Application 10/080,794 and has the sequence of Seq. ID. NO. 4.

Fig. 1 shows the results when 607B melanoma cells were treated with either the antisense oligonucleotide at concentrations of 100, 250 or 500 nM, or a scrambled control at a concentration of 100 nM on two consecutive days. Lipofectin™ (lip) without oligonucleotide was used as a control. Cells numbers in 96 well plates were measured photometrically using MTS (Cell Titer 96™, Pierce). As shown, cell counts in the presence

of antisense treated wells at 250 and 500 nM are significantly reduced.

Fig. 2 provides a graphic representations of clusterin expression in 518A2 cells after treatment with cisplatin and either an antisense oligonucleotide or a scrambled control. Lip is a Lipofectin control without oligonucleotide. Detection was performed using an antibody directed against clusterin.

The results in showed that in human melanoma cells clusterin is expressed at significantly higher levels than in human melanocytes in all but one cell line tested. The antisense inhibitor (MOE modification of Seq. ID. NO. 4) led to a dose dependent down-regulation of clusterin as shown by RT-PCR on the mRNA level and by western-blot on the protein level as compared to the scrambled mismatch control. This down-regulation led to an increase in apoptotic cell death by antisense treatment alone. In one melanoma cell line (607B) this alone was sufficient to lead to complete cell death. (Fig. 1) In another melanoma cell line the surviving cells showed increased sensitivity to an consecutive treatment with the cytotoxic drug cisplatin as compared to cells treated with a control-mismatch oligonucleotide (Figure 2).

Example 2

Mel Juso melanoma cells stably tranfected with either an empty control vector (Neo) or a vector directing overexpression of clusterin were grown in medium containing 10 ΦM cisplatin. Cell survival was measured using the Cell-titer 96 kits from Promega. The results are summarized in Figure 3. As shown, overexpression of clusterin dramatically enhanced cell survival, or said differently, reduced the effectiveness of the chemotherapy agent.

CLAIMS

1. A method for treatment of melanoma in a mammalian subject, comprising the step of administering to the subject a therapeutic agent effective to reduce the effective amount of clusterin in the melanoma cells.

2. The method of claim 1, wherein the therapeutic agent is an antisense oligodeoxynucleotide effective to reduce the effective amount of clusterin in the melanoma cells.

3. The method of claim 2, wherein the antisense oligodeoxynucleotide spans either the translation initiation site or the termination site.

4. The method of claim 3, wherein the antisense oligodeoxynucleotide is modified to enhance in vivo stability relative to an unmodified oligodeoxynucleotide of the same sequence.

5. The method of claim 4, wherein the modification is a (2'-O-(2-methoxyethyl) modification.

6. The method of any of claims 1-5, wherein the antisense oligodeoxynucleotide consists essentially of an oligodeoxynucleotide selected from the group consisting of Seq. ID. Nos. 2 to 19.

7. The method of claim 6, wherein the antisense oligodeoxynucleotide consists essentially of an oligodeoxynucleotide consisting of Seq. ID. No. 4.

8. The method of claim 7, wherein the oligonucleotide has a phosphorothioate backbone throughout, the sugar moieties of nucleotides 1-4 and 18-21, the "wings", bear 2'-O-methoxyethyl modifications and the remaining nucleotides are 2'-deoxynucleotides.

9. The method of claim 1, wherein the therapeutic agent is an RNA molecule effective to reduce the effective amount of clusterin in the melanoma cells by an RNAi mechanism.

10. The method of claim 9, wherein the RNA molecule consists essentially of an oligodeoxynucleotide selected from the group consisting of Seq. ID. Nos.20 to 25.

11. Use of a composition comprising a therapeutic agent effective to reduce the effective amount of clusterin in the melanoma cells for the formulation of a pharmaceutical composition for use in treatment of melanoma.

12. Use of claim 11, wherein the therapeutic agent is an is an antisense oligodeoxynucleotide effective to reduce the effective amount of clusterin in the melanoma cells.

13. Use of claim 12, wherein the antisense oligodeoxynucleotide spans either the translation initiation site or the termination site.

14. Use of claim 13, wherein the antisense oligodeoxynucleotide is modified to enhance in vivo stability relative to an unmodified oligodeoxynucleotide of the same sequence.

15. Use of claim 14, wherein the modification is a (2'-O-(2-methoxyethyl) modification.

16. Use of any of claims 12-15, wherein the antisense oligodeoxynucleotide consists essentially of an oligodeoxynucleotide selected from the group consisting of Seq. ID. Nos. 2 to 19.

17. Use of claim 16, wherein the antisense oligodeoxynucleotide consists essentially of an oligodeoxynucleotide consisting of Seq. ID. No. 4.

18. Use of claim 17, wherein the oligonucleotide has a phosphorothioate backbone throughout, the sugar moieties of nucleotides 1-4 and 18-21, the "wings", bear 2'-O-methoxyethyl modifications and the remaining nucleotides are 2'-deoxynucleotides.

19. Use of claim 11, wherein the therapeutic agent is an RNA molecule effective to reduce the effective amount of clusterin in the melanoma cells by an RNAi mechanism.

20. Use of claim 19, wherein the RNA molecule consists essentially of an oligodeoxynucleotide selected from the group consisting of Seq. ID. Nos.20 to 43.

21. A method for regulating expression of bcl-xL in a subject or cell line comprising administering to the subject or cell line an agent effective to modulate the amount of clusterin expression.

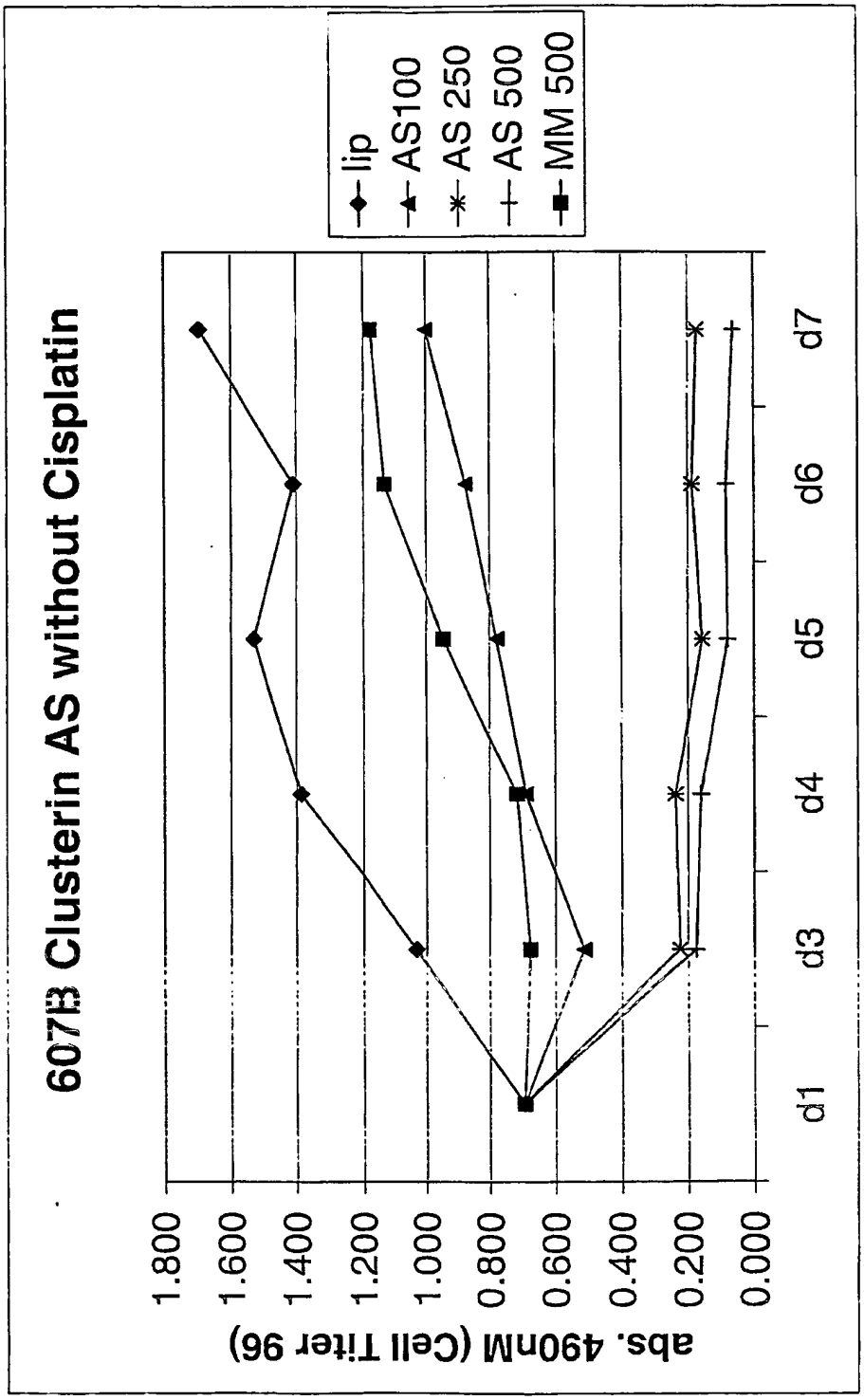

607B Clusterin AS without Cisplatin
abs. 490nM (Cell Titer 96)
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
d1
d3
d4
d5
d6
d7
lip
AS100
AS 250
AS 500
MM 500

Fig. 1

518A2 Dose Relation Curve 24 hrs after addition of Cisplatin

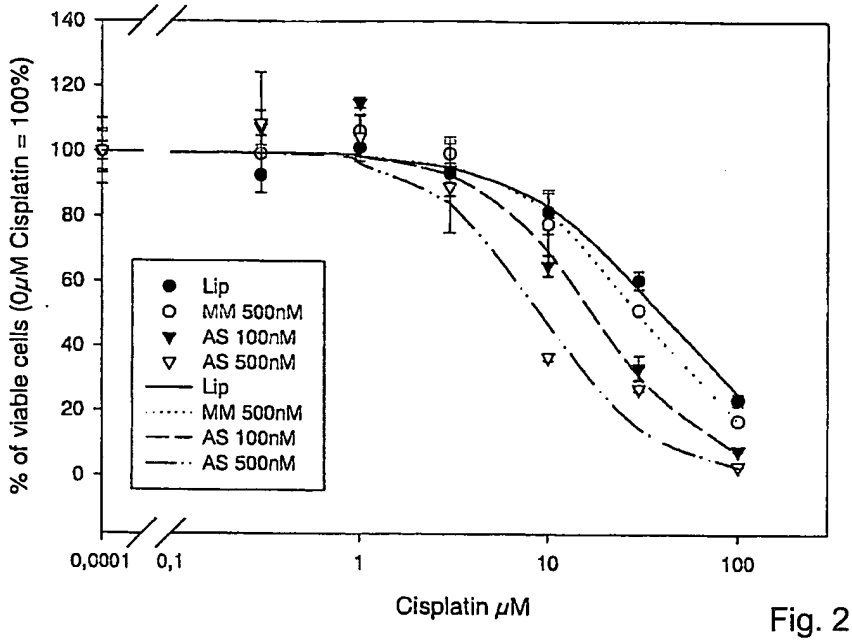


Fig. 2

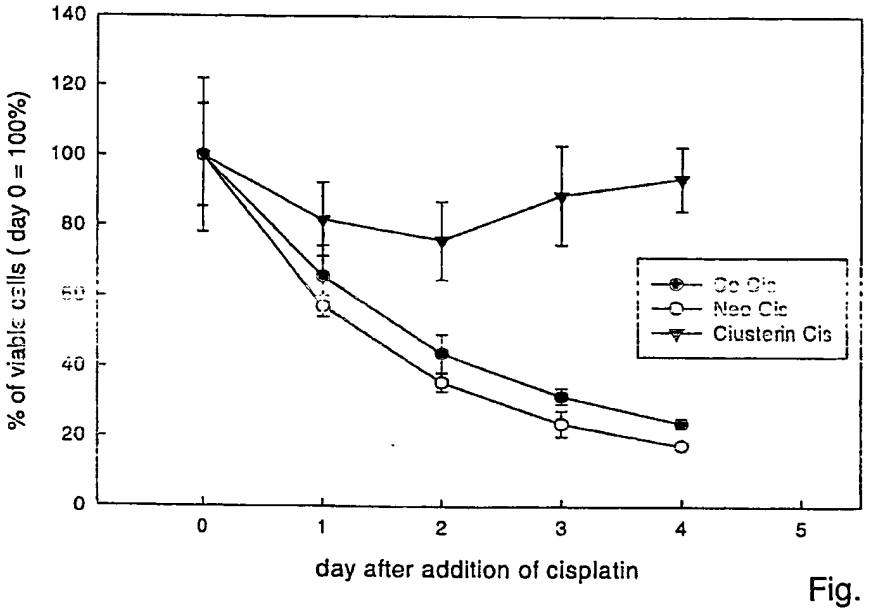


Fig. 3

SEQUENCE LISTING

<110> THE UNIVERSITY OF BRITISH COLUMBIA
GLEAVE, Martin
JANSEN, Burkhard

<120> TREATMENT OF MELANOMA BY REDUCTION IN CLUSTERIN LEVELS

<130> 49101-4

<140> NOT YET ASSIGNED
<141> 2003-08-21

<150> US 60/405,193
<151> 2002-08-21

<150> US 60/319,748
<151> 2002-12-02

<150> US 60/408,152
<151> 2002-09-03

<150> US 60/473,387
<151> 2003-05-20

<160> 43

<170> PatentIn version 3.2

<210> 1
<211> 1676
<212> DNA
<213> Homo sapiens

<400> 1
gaattccgcc gctgaccgag gcgtgcaaag actccagaat tggaggcatg atgaagactc 60
tgctgctgtt tgtggggctg ctgctgacct gggagagtgg gcaggtcctg ggggaccaga 120
cggtctcaga caatgagctc caggaaatgt ccaatcaggg aagtaagtac gtcaataagg 180
aaattcaaaa tgctgtcaac ggggtgaaac agataaagac tctcatagaa aaaacaaacg 240
aagagcgcaa gacactgctc agcaacctag aagaagccaa gaagaagaaa gaggatgccc 300
taaatgagac caggcaatcc gagacaaagc tgaaggagct cccaggagtg tgcaatgaga 360
ccatgatggc cctcrgggaa gagtgtaagc cctgccrgaa acagacctgc atgaagtrct 420
acgcacgcgt ctgcagaagt ggctcaggcc tggttggccg ccagcttgag gagttcctga 480
accagagctc gcccttctac ttctggatga atggtgaccg catcgactcc ctgctggaga 540
acgaccggca gcagacgcac atgctggatg tcatgcagga ccacttcagc cgcgcgtcca 600
gcatcataga cgagctcttc caggacaggt tcttcacccg ggagccccag gatacctacc 660
actacctgcc cttcagcctg ccccaccgga ggcctcactt cttctttccc aagtcccgca 720
tcgtccgcag cttgatgccc ttctctccgt acgagccccc gaacttccac gccatgttcc 780
agcccttcct tgagatgata cacgaggctc agcaggccat ggacatccac ttccacagcc 840
cggccttcca gcacccgcca acagaattca tacgagaagg cgacgatgac cggactgtgt 900
gccgggagat ccgccacaac tccacgggct gcctgcggat gaaggaccag tgtgacaagt 960
gccgggagat cttgtctgtg gactgttcca ccaacaaccc ctcccaggct aagctgcggc 1020
gggagctcga cgaatccctc caggtcgctg agaggttgac caggaaatac aacgagctgc 1080
taaagtccta ccagtggaag atgctcaaca cctcctcctt gctggagcag ctgaacgagc 1140
agtttaactg ggtgtcccgg ctggcaaacc tcacgcaagg cgaagaccag tactatctgc 1200
gggtcaccac ggtggcttcc cacacttctg actcggacgt tccttccggt gtcactgagg 1260
tggtcgtgaa gctctttgac tctgatccca tcactgtgac ggtccctgta gaagtctcca 1320
ggaagaaccc taaatttatg gagaccgtgg cggagaaagc gctgcaggaa taccgcaaaa 1380
agcaccggga ggagtgagat gtggatgttg cttttgcacc ttacgggggc atcttgagtc 1440
cagctccccc caagatgagc tgcagccccc cagagagagc tctgcacgtc accaagtaac 1500

caggccccag cctccaggcc cccaactccg cccagcctct ccccgctctg gatcctgcac 1560
tctaacactc gactctgctg ctcatgggaa gaacagaatt gctcctgcat gcaactaatt 1620
caataaaact gtcttgtgag ctgaaaaaaa aaaaaaaaaa aaaaaaaaag gaattc 1676

<210> 2
<211> 21
<212> DNA
<213> Mus musculus

<400> 2
gcacagcagg agaatcttca t 21

<210> 3
<211> 21
<212> DNA
<213> Homo sapiens

<400> 3
tggagtcttt gcacgcctcg g 21

<210> 4
<211> 21
<212> DNA
<213> Homo sapiens

<400> 4
cagcagcaga gtcttcatca t 21

<210> 5
<211> 21
<212> DNA
<213> Homo sapiens

<400> 5
attgtctgag accgtctggt c 21

<210> 6
<211> 21
<212> DNA
<213> Homo sapiens

<400> 6
ccttcagctt tgtctctgat t 21

<210> 7
<211> 21
<212> DNA
<213> Homo sapiens

<400> 7
agcagggagt cgatgcggtc a 21

<210> 8
<211> 21
<212> DNA
<213> Homo sapiens

<400> 8
atcaagctgc ggacgatgcg g 21

<210> 9
<211> 21
<212> DNA
<213> Homo sapiens

<400> 9
gcaggcagcc cgtggagttg t 21

<210> 10
<211> 21
<212> DNA
<213> Homo sapiens

<400> 10
ttcagctgct ccagcaagga g 21

<210> 11
<211> 21
<212> DNA
<213> Homo sapiens

<400> 11
aatttagggt tcttcctgga g 21

<210> 12
<211> 21
<212> DNA
<213> Homo sapiens

<400> 12
gctgggcgga gttgggggcc t 21

<210> 13
<211> 17
<212> DNA
<213> Homo sapiens

<400> 13
ggtgtagacg ccgcacg 17

<210> 14
<211> 16
<212> DNA
<213> Homo sapiens

<400> 14
gcagcgcagc ccctgg 16

<210> 15
<211> 22
<212> DNA
<213> Homo sapiens

<400> 15
gcagcagccg cagcccggct cc 22

<210> 16
<211> 18
<212> DNA
<213> Homo sapiens

<400> 16
agccgcagcc cggctcct 18

<210> 17
<211> 20
<212> DNA
<213> Homo sapiens

<400> 17
cagcagccgc agcccggctc 20

<210> 18
<211> 20
<212> DNA
<213> Homo sapiens

<400> 18
gcagcagccg cagcccggct 20

<210> 19
<211> 20
<212> DNA
<213> Homo sapiens

<400> 19
agcagccgca gcccggctcc 20

<210> 20
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 20
ccagagcucg cccuucuact t 21

<210> 21
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 21
guagaagggc gagcucuggt t 21

<210> 22
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 22
gaugcucaac accuccucct t 21

<210> 23
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 23
ggaggaggug uugagcauct t 21

<210> 24
<211> 19
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 24
uaauucaaca aaacugutt 19

<210> 25
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 25
gacaguuuua uugaauuagt t 21

<210> 26
<211> 19
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 26
uaauucaaca aaacugutt 19

<210> 27
<211> 19
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 27
acaguuuugu ugaauuatt 19

<210> 28
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 28
augaugaaga cucugcugct t 21

<210> 29
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 29
gcagcagagu cuucaucaut t 21

<210> 30
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 30
ugaaugaagg gacuaaccug tt 22

<210> 31
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 31
cagguuaguc ccuucauuca tt 22

<210> 32
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 32
cagaaauaga caaagugggg tt 22

<210> 33
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 33
ccccacuuug ucuauuucug tt 22

<210> 34
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 34
acagagacua agggaccaga tt 22

<210> 35
<211> 22
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 35
acagagacua agggaccaga tt 22

<210> 36
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 36
ccagagcucg cccuucuact t 21

<210> 37
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 37
guagaagggc gagcucuggt t 21

<210> 38
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 38
gucccgcauc guccgcagct t 21

<210> 39
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 39
gcugcggacg augcgggact t 21

<210> 40
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 40
cuaauucaau aaaacuguct t 21

<210> 41
<211> 21
<212> DNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 41
gacaguuuua uugaauuagt t 21

<210> 42
<211> 19
<212> RNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 42
augaugaaga cucugcugc 19

<210> 43
<211> 19
<212> RNA
<213> artificial

<220>
<223> RNAi for human clusterin

<400> 43
gcagcagagu cuucaucau 19

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01276

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/11 A61K31/712

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02 22635 A (MONIA BRETT P ;FREIER SUSAN M (US); ISIS PHARMACEUTICALS INC (US)) 21 March 2002 (2002-03-21)	
A	WO 00 49937 A (UNIV BRITISH COLUMBIA ;GLEAVE MARTIN (CA); RENNIE PAUL S (CA); MIY) 31 August 2000 (2000-08-31) cited in the application	
A	US 6 172 216 B1 (NICKOLOFF BRIAN J ET AL) 9 January 2001 (2001-01-09)	

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2003	22/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Marinoni, J-C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ZELLWEGER TOBIAS ET AL: "Antitumor activity of antisense clusterin oligonucleotides is improved in vitro and in vivo by incorporation of 2'-O-(2-methoxy)ethyl chemistry" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 298, no. 3, September 2001 (2001-09), pages 934-940, XP002262318 ISSN: 0022-3565	
A	WILSON M R M R ET AL: "Clusterin is a secreted mammalian chaperone" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 25, no. 3, March 2000 (2000-03), pages 95-98, XP004202536 ISSN: 0968-0004	
A	WONG P ET AL: "MOLECULAR CHARACTERIZATION OF HUMAN TRPM-2/CLUSTERIN, A GENE ASSOCIATED WITH SPERM MATURATION, APOPTOSIS AND NEURODEGENERATION" EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, vol. 221, no. 3, 1994, pages 917-925, XP001146404 ISSN: 0014-2956	
A	JONES STEVE E ET AL: "Clusterin" INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, vol. 34, no. 5, May 2002 (2002-05), pages 427-431, XP002262319 ISSN: 1357-2725	
A	GLEAVE MARTIN E ET AL: "Use of antisense oligonucleotides targeting the antiapoptotic gene, clusterin/testosterone-repressed prostate message 2, to enhance androgen sensitivity and chemosensitivity in prostate cancer" UROLOGY, vol. 58, no. 2A Supplement, August 2001 (2001-08), pages 39-49, XP002262320 ISSN: 0090-4295	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSENBERG M E ET AL: "CLUSTERIN: PHYSIOLOGIC AND PATHOPHYSIOLOGIC CONSIDERATIONS" INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, EXETER, GB, vol. 27, no. 7, 1995, pages 633-645, XP001002844 ISSN: 1357-2725 ---	
A	MIYAKE H ET AL: "Novel therapeutic strategy for advanced prostate cancer using antisense oligodeoxynucleotides targeting anti-apoptotic genes upregulated after androgen withdrawal to delay androgen-independent progression and enhance chemosensitivity" INTERNATIONAL JOURNAL OF UROLOGY 2001 AUSTRALIA, vol. 8, no. 7, 2001, pages 337-349, XP002262321 ISSN: 0919-8172 ---	
A	GLEAVE M E ET AL: "Targeting anti-apoptotic genes upregulated by androgen withdrawal using antisense oligonucleotides to enhance androgen- and chemo-sensitivity in prostate cancer" INVESTIGATIONAL NEW DRUGS 2002 UNITED STATES, vol. 20, no. 2, 2002, pages 145-158, XP009021411 ISSN: 0167-6997 ---	
A	MIYAKE H ET AL: "ANTISENSE TRPM-2 OLIGODEOXYNUCLEOTIDES CHEMOSENSITIZE HUMAN ANDROGEN-INDEPENDENT PC-3 PROSTATE CANCER CELLS BOTH IN VITRO AND IN VIVO" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 6, no. 5, May 2000 (2000-05), pages 1655-1663, XP000960694 ISSN: 1078-0432 ---	
A	SENSIBAR J A ET AL: "PREVENTION OF CELL DEATH INDUCED BY TUMOR NECROSIS FACTOR ALPHA IN LNCAP CELLS BY OVEREXPRESSION OF SULFATED GLYCOPROTEIN-2 (CLUSTERIN)" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 55, June 1995 (1995-06), pages 2431-2437, XP002930082 ISSN: 0008-5472 cited in the application ---	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZANGEMEISTER-WITTKE U ET AL: "A novel bispecific antisense oligonucleotide inhibiting both bcl-2 and bcl-xL expression efficiently induces apoptosis in tumor cells" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 6, no. 6, June 2000 (2000-06), pages 2547-2555, XP002241562 ISSN: 1078-0432 ---	
A	MIYAKE H ET AL: "TESTOSTERONE-REPRESSED PROSTATE MESSAGE-2 IS AN ANTIAPOPTOTIC GENE INVOLVED IN PROGRESSION TO ANDROGEN INDEPENDENCE IN PROSTATE CANCER" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 60, 1 January 2000 (2000-01-01), pages 170-176, XP002907064 ISSN: 0008-5472 ---	
A	ZELLWEGER T ET AL: "CHEMOSENSITIZATION OF HUMAN RENAL CELL CANCER USING ANTISENSE OLIGONUCLEOTIDES TARGETING THE ANTIAPOPTOTIC GENE CLUSTERIN" NEOPLASIA, DOYMA, BARCELONA,, ES, vol. 3, no. 4, July 2001 (2001-07), pages 360-367, XP009004604 ISSN: 0212-9787 ---	
A	GLEAVE M ET AL: "ANTISENSE THERAPY: CURRENT STATUS IN PROSTATE CANCER AND OTHER MALIGNANCIES" CANCER AND METASTASIS REVIEWS, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 21, 1992, pages 79-92, XP001147871 ISSN: 0167-7659 ---.	
A	MIYAKE H ET AL: "Synergistic chemosensitization and inhibition of tumor growth and metastasis by the antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model." CLINICAL CANCER RESEARCH: AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. UNITED STATES DEC 2001, vol. 7, no. 12, December 2001 (2001-12), pages 4245-4252, XP002263075 ISSN: 1078-0432 ---	
P,A	WO 03 062421 A (UNIV BRITISH COLUMBIA ;GLEAVE MARTIN (CA); SIGNAEVSKY MAXIM (CA)) 31 July 2003 (2003-07-31) ---	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	GLEAVE MARTIN ET AL: "Antisense targets to enhance hormone and cytotoxic therapies in advanced prostate cancer." CURRENT DRUG TARGETS. NETHERLANDS APR 2003, vol. 4, no. 3, April 2003 (2003-04), pages 209-221, XP009021409 ISSN: 1389-4501 ---	
E	WO 03 072591 A (UNIV BRITISH COLUMBIA ;GLEAVE MARTIN (CA); RENNIE PAUL S (CA); MIY) 4 September 2003 (2003-09-04) the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/01276

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-10,21 all partially
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-5, 9, 11-15, 19, 21 all partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 1-10 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-10,21 all partially

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

---

Continuation of Box I.2

Claims Nos.: 1-5, 9, 11-15, 19, 21 all partially

Present claims 1, 11 and 21 refer to an undefined agent defined by reference to a desirable characteristic or property, namely that it is "effective to reduce the effective amount of clusterin in the melanoma cell" (claims 1 and 11) or that it is "effective to modulate the amount of clusterin expression" (claim 21).

The claims cover all "agents" having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such "agents". In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the "agent" by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts where the agent is defined as being an antisense oligonucleotide or a RNA molecule hybridizing to and reducing the expression of the clusterin gene (claims 6-8, 10, 16-18, 20 and 21).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 03/01276

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0222635	A	21-03-2002	US	6383808 B1	07-05-2002
			AU	9070601 A	26-03-2002
			EP	1325019 A1	09-07-2003
			WO	0222635 A1	21-03-2002
WO 0049937	A	31-08-2000	AU	767133 B2	30-10-2003
			AU	3606400 A	14-09-2000
			CA	2371814 A1	31-08-2000
			EP	1163254 A2	19-12-2001
			HU	0200093 A2	29-05-2002
			JP	2003503309 T	28-01-2003
			NO	20014058 A	22-10-2001
			NZ	513757 A	28-09-2001
			WO	0049937 A2	31-08-2000
			US	2003166591 A1	04-09-2003
			US	2002128220 A1	12-09-2002
US 6172216	B1	09-01-2001	AU	755515 B2	12-12-2002
			AU	6271099 A	26-04-2000
			CA	2345354 A1	13-04-2000
			EP	1119579 A1	01-08-2001
			JP	2002526093 T	20-08-2002
			WO	0020432 A1	13-04-2000
			US	6210892 B1	03-04-2001
			US	2003191300 A1	09-10-2003
			US	6214986 B1	10-04-2001
			US	2001007025 A1	05-07-2001
WO 03062421	A	31-07-2003	WO	03062421 A1	31-07-2003
			US	2003158143 A1	21-08-2003
WO 03072591	A	04-09-2003	US	2003166591 A1	04-09-2003
			WO	03072591 A1	04-09-2003